The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease

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Summary

The study is designed to investigate the changes and roles of T helper type 17/regulatory T cells (Th17/T_{reg}) in the immunological pathogenesis of Kawasaki disease (KD). In addition, we explore the alteration and significance of Th17 cells in patients with intravenous immune globulin-resistant KD. Real-time polymerase chain reaction (PCR) was used to evaluate the mRNA levels of interleukin (IL)-17A/F, retinoic acid-related orphan receptor (ROR)-γt and forkhead box P3 (FoxP3) in CD4-positive cells. The proportions of Th17 cells and $CD4^+CD25^+FoxP3^{high}$ T_{regs} were analysed by flow cytometry. Plasma cytokine [IL-17A, IL-6, IL-23 and transforming growth factor (TGF)-β] concentrations were measured by sandwich enzyme-linked immunosorbent assay. Our data demonstrate that Th17 proportions and expression levels of cytokines (IL-17, IL-6 and IL-23) and transcription factors (IL-17A/F, ROR-γt) were up-regulated significantly, while T_{reg} proportions and expression levels of T_{reg} transcription factor (FoxP3) were down-regulated significantly in children with acute KD ($P < 0.01$). Compared with the sensitive group, the Th17 proportions were up-regulated significantly during the acute phase in immune globulin-resistant KD ($P < 0.01$). The plasma IL-17A, IL-6 and IL-23 concentrations in patients with KD were significantly higher compared with the concentrations in normal controls (NC) and infectious disease (ID). Plasma TGF-β concentrations were markedly lower in the KD group than the NC and ID groups ($P < 0.05$). These results suggest that Th17/T_{reg} cells imbalance exists in the patients with KD. Th17/T cells imbalance may be important factors causing disturbed immunological function and resulting in immunoglobulin-resistant KD.

Keywords: immunoglobulin, interleukin-6, -17, -23, Kawasaki disease, Th17 cells, T_{reg} cells

Introduction

Kawasaki disease (KD) is an acute vasculitis that affects infants and children, and is the leading cause of acquired heart disease in the paediatric age group. The immunopathogenesis of KD needs to be investigated. A great many studies have found that the levels of many proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-6 are elevated in acute KD, but the mechanisms resulting in aberrant immune function or over-expression of proinflammatory cytokines are not clear [1–5].

Recently, $CD4^+CD25^+$ regulatory T cells (T_{reg}) and T helper type 17 (Th17) cells have been described as two distinct subsets from Th1 and Th2 cells. T_{reg} cells expressing the forkhead/winged helix transcription factor P3 (FoxP3) have an anti-inflammatory role and maintain tolerance to self-components by contact-dependent suppression or releasing anti-inflammatory cytokines [IL-10 and transforming growth factor (TGF)-β1] [6], while Th17 cells expressing retinoic acid-related orphan receptor γt (ROR-γt) play critical roles in the development of autoimmunity and allergic reactions by producing IL-17 [7]. IL-17A and IL-17F produced by Th17 cells have proinflammatory properties and act on a broad range of cell types to induce the expression of cytokines [IL-6, TNF-α, IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF)], chemokines (cxcl1, cxcl10) and metalloproteinases, thus perpetuating inflammation of the tissues [8,9]. As there are few data available on

Table 1. Characteristics of patients with Kawasaki disease (KD).

	KD
Age (months)	28·0 ± 16·4
Sex (male/female)	28/17
Duration of fever (days), mean (s.d.)	9·76 (6·4)
Rash	42 (93·3%)
Lymphadenopathy	44 (97·8%)
Conjunctival congestion	42 (93·3%)
Oral mucosal changes	43 (95·5%)
Peeling	42 (93·3%)
Arthritis	12 (26·7%)
Coronary dilatation	15 (33·3%)
Thrombocytosis	42 (93·3%)
Pyuria	5 (11%)
Jaundice	1 (2%)
Giant peripheral aneurysm	0

s.d.: standard deviation.

Th17 T_{reg} cells in KD, we investigate the frequency of Th17 and T_{reg} cells in peripheral blood and the cytokines affecting Th17 and T_{reg} differentiation in plasma to evaluate whether the Th17/T_{reg} balance was disrupted in patients with KD.

Materials and methods

Forty-five children with acute febrile stage of KD (28 males and 17 females; mean age: 25·2 ± 14·4 months; age range: 8 months–4·7 years), age-matched control subjects including 18 patients with active infections disease (ID) (six with measles, seven with influenza, one with rotavirus, two with adenovirus and two with Epstein–Barr virus) (10 males and eight females; mean age: 15·8 ± 13·1 months; age range: 8 months–3 years) and 20 age-matched normal controls (NC) (11 males and nine females; mean age: 24·0 ± 14·4 months; age range: 1–4·5 years) were enrolled into this study (Table 1). The patients described above comprise the sensitive group who received intravenous immunoglobulin (IVIG) therapy. In addition, we selected 10 children (six

males and four females; mean age: 15·0 ± 12·8 months; age range: 5 months–2·5 years) who were IVIG-resistant KD (patients were defined as resistant if their fever continued > 24 h after IVIG, or rose again within 48 h) to evaluate the effect of Th17 cells on IVIG treatment response. Informed consent was obtained from their parents and the study was approved by the medical ethics hospital committee. The diagnosis was made according to the clinical criteria of the Kawasaki Disease Research Committee of Japan. Blood samples from patients with KD before and 10 days after IVIG therapy were collected. Blood samples were analysed immediately without stimulation of mitogens or culture *in vitro* unless particularly indicated. All patients with KD received two-dimensional echocardiographic examination. Coronary artery lesion (CAL) was defined by internal diameter of artery > 3·0 mm (< 5 years); > 4·0 mm (≥ 5 years) or coronary artery aneurysms. Patients with KD were divided into the KD-CAL+ group (15) and the KD-CAL− group (30) according to the echocardiographic examination results (Tables 2 and 3).

Blood samples

Venous blood (5 ml) was taken from patients with KD, normal controls and patients with ID using ethylenediamine tetraacetic acid (EDTA) Na_2 as anti-coagulant. Peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll density gradient for flow cytometric analysis. Plasma was obtained after centrifugation and stored at −80°C for measurement of the enzyme-linked immunosorbent assay (ELISA). CD4+ T cells were isolated immediately from peripheral blood of the children participating in the study, according to the manufacturer's instructions for microbeads (111·49D; Dynal, Oslo, Norway). Purified cells were identified as > 97% with flow cytometry (FCM) (Fig. 1), while results of cell activity were > 95% by 0·05% trypan blue staining.

Table 2. Clinical data of patients with Kawasaki disease–coronary artery lesion (KD-CAL+) and KD-CAL−.

| Groups	n	Age (months)	WBC (×10^9/l)	PLT (×10^9/l)	Hb (g/l)	CRP (mg/l)	ESR (mm/h)	Alb (g/l)	LDH (IU/l)
KD-CAL+	15	21·0 ± 15·6	14·8 ± 3·7	415 ± 87	109·2 ± 10·8	86·2 ± 24·6	50·6 ± 18·4	36·8 ± 3·8	185·6 ± 51·7
KD-CAL−	30	26·2 ± 12·4	14·1 ± 4·6	350 ± 66*	107·3 ± 9·6	66·5 ± 21·3*	37·2 ± 9·5*	38·0 ± 2·4	179·1 ± 53·3

Values are expressed as mean ± standard deviation. WBC: white blood cell; PLT: platelet; Hb: haemoglobin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Alb: serum albumin; LDH: lactate dehydrogenase. KD-CAL+ *versus* KD-CAL−: *$P < 0.05$.

Table 3. Clinical data of patients with intravenous immunoglobulin (IVIG)-sensitive and IVIG-resistant Kawasaki disease.

Groups	n	Age (months)	WBC (×10^9/l)	PLT (×10^9/l)	Hb (g/l)	CRP (mg/l)	ESR (mm/h)	Alb (g/l)	LDH (IU/l)
IVIG-sensitive	35	25·2 ± 14·4	14·3 ± 3·6	361 ± 51	108·5 ± 10·2	78·4 ± 31·7	40·7 ± 15·2	37·0 ± 3·7	182·1 ± 50·8
IVIG-resistant	10	15·0 ± 12·8*	19·7 ± 4·5*	386 ± 76	92·8 ± 5·7*	98·8 ± 20·6	48·4 ± 12·7	24·4 ± 2·5*	352·4 ± 62·5*

Values are expressed as mean ± standard deviation. WBC: white blood cell; PLT: platelet; Hb: haemoglobin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Alb: serum albumin; LDH: lactate dehydrogenase. IVIG-sensitive *versus* IVIG-resistant: *$P < 0.05$.
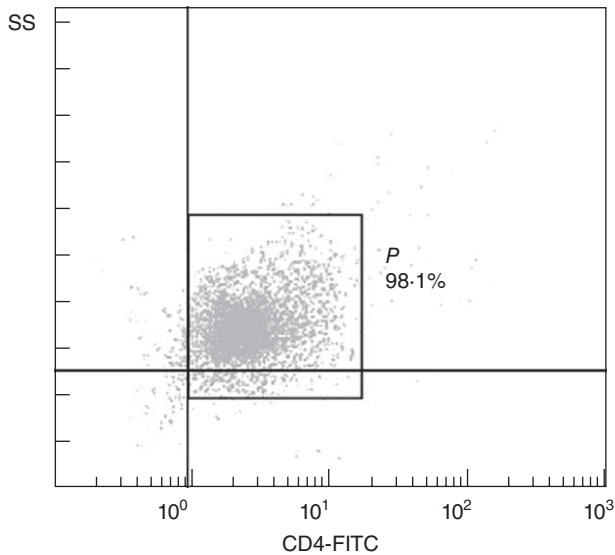
Fig. 1. Cell purity detected with flow cytometry after isolation with magnetic affinity cell sorting.

Total RNA extraction and cDNA synthesis

Total RNA from CD4$^+$ T cells was prepared using the Versagene RNA Kit (0050C; Gentra, Minneapolis, MN, USA), according to the manufacturer's instructions. DNase I (0050D; Gentra) was used to eliminate the trace DNA during extraction. Isolated total RNA integrity was verified by an average optical density (OD) OD$_{260}$/OD$_{286}$ absorption to cDNA with oligodeoxythymidylic acid (oligo-dT) primer, using RevertAid™ H Minus Moloney murine leukaemia virus (MMLV) reverse transcriptase (K1632$^#$; Fermentas, Vilnius, Lithuania). Negative control samples (no first-strand synthesis) were prepared by performing reverse transcription reaction in the absence of reverse transcriptase.

LightCycler real-time polymerase chain reaction (PCR)

The cDNA levels of IL-17A/F, ROR-γt and FoxP3 and other genes were quantitated by real-time PCR using Quantitect™

SYBR green PCR Kit (204143; Qiagen, Hilden, Germany) and LightCycler II (Roche, Mannhein, Germany) according to the manufacturer's maximum method was performed for CP (cross point) determination using LightCycler Software version 3·5 (Roche Molecular Biochemicals, Mannhein, Germany). After normalization with Relative Quantification Software 1·0 (Roche Molecular Biochemicals), cDNA levels were identified as: target genes/β-actin (shown in Table 4).

Flow cytometric analysis of Th17 and T$_{reg}$

For analysis of Th17 and T$_{reg}$, PBMCs were suspended at a density of 2×106 cells/ml into tubes and washed once in phosphate-buffered saline (PBS). For Th17 analysis, the cells were incubated with phycoerythrin (PE) anti-human CD4 (eBioscience, San Diego, CA, USA) at 4°C for 20 min. For T$_{reg}$ analysis, the cells were incubated with peridinin chlorophyll (PerCP) anti-human CD4 and fluorescein isothiocyanate (FITC) anti-human CD25. After surface staining, the cells were stained with FITC anti-human IL-17A for Th17 detection or PE anti-human FoxP3 for T$_{reg}$ detection after fixation and permeabilization according to the manufacturer's instructions. Isotype controls were given to enable correct compensation and confirm antibody specificity. All the antibodies were from eBioscience. Stained cells were analysed by flow cytometric analysis using an Epics-XL4 cytometer equipped with EXPO32 ADC software (Beckman Coulter, San Diego, CA, USA).

ELISA detection of plasma IL-17, IL-6, IL-23 and TGF-β

The plasma levels of IL-17, IL-6, IL-23 and TGF-β were measured by ELISA using an ELX-800 microplate reader (Biotek Corporation, Winooski, VT, USA), following the manufacturer's instructions (eBioscience). All samples were measured in duplicate.

Statistical analysis

The software used for statistical analysis was SPSS for Windows version 13·0 (SPSS, Chicago, IL, USA). Data are

Table 4. Primer for real-time polymerase chain reaction.

Gene	mRNA numbers	Sequence	Annealing temperature (°C)	Product size base pairs
IL-17A	NM_002190	Sense: 5′-CAG ATT ACT ACA ACC GAT CC-3′	57	140
		Anti-sense: 5′-CAT GTG GTA GTC CAC GTT CC-3′		
IL-17F	NM_052872	Sense: 5′-CCG TTC CCA TCC AGC AAG AG-3′	58	122
		Anti-sense: 5′-ACA GTC ACC AGC ACC TTC TC-3′		
ROR-γt	NM_005060	Sense: 5′-GTG CTG GTT AGG ATG TGC CG 3′	58	135
		Anti-sense: 5′-GTG GGA GAA GTC AAA GAT GGA-3′		
FoxP3	NM_014009	Sense: 5′-GTG GCA TCA TCC GAC AAG G -3′	58	166
		Anti-sense: 5′-TGT GGA GGA ACT CTG GGA AT-3′		
β-actin	NM_001101	Sense: 5′-GAG CTA CGA GCT GCC TGA CG-3′	56–61	120
		Anti-sense: 5′-GTA GTT TCG TGG ATG CCA CAG-3′		

IL: interleukin; FoxP3: forkhead box P3; ROR-γt: retinoic acid-related orphan receptor γt.

Table 5. Expression of cytokines associated with T helper type 17 (Th17) and regulatory T cell (T$_{reg}$) differentiation in patients with Kawasaki disease.

Cytokines	NC group ($n = 20$)	ID group ($n = 18$)	KD group ($n = 45$)	KD-CAL$^+$ group ($n = 15$)	KD-CAL$^-$ group ($n = 30$)	KD^{IVIG} group ($n = 45$)
IL-17A (pg/ml)	7·5 ± 2·6	15·2 ± 4·7[a]	30·3 ± 10·2[b]	35·5 ± 10·7	28·3 ± 8·6[c]	26·8 ± 8·8[e]
IL-6 (pg/ml)	12·2 ± 4·0	21·7 ± 6·0[a]	71·6 ± 21·9[b]	80·8 ± 26·2	65·6 ± 20·2[c]	63·5 ± 17·1[e]
IL-23 (pg/ml)	118 ± 37	205 ± 68[a]	420 ± 114[b]	496 ± 184	315 ± 104[c]	370 ± 127[e]
TGF-β (pg/ml)	836 ± 257	813 ± 239[a]	265 ± 68[b]	280 ± 83	276 ± 78[d]	306 ± 154[e]

Values are expressed as mean ± standard deviation. IL: interleukin; TGF: transforming growth factor. Infectious disease (ID) *versus* normal controls (NC): [a]$P < 0.05$; ID *versus* KD: [b]$P < 0.05$; Kawasaki disease–coronary artery lesion (KD-CAL$^+$) *versus* KD-CAL$^-$: [c]$P < 0.05$, [d]$P > 0.05$; KD *versus* KD^{IVIG}: [e]$P > 0.05$.

presented as mean ± standard deviation (s.d.). Differences between the values were determined using Student's *t*-test. A value of $P < 0.05$ was regarded as a significant difference.

Results

Circulating Th17 and T$_{reg}$ frequencies

As shown in Fig. 2, compared with healthy controls the Th17 cells proportions were up-regulated significantly in the ID group ($1.53 \pm 0.40\%$ *versus* $0.28 \pm 0.15\%$, $P < 0.01$). The frequencies of Th17 (CD4$^+$IL-17$^+$/CD4$^+$ T cells) were markedly higher in patients with KD than those in the ID group ($2.72 \pm 0.71\%$ *versus* $1.53 \pm 0.40\%$, $P < 0.01$) and the proportions of Th17 cells had a tendency to decrease after treatment with IVIG ($2.72 \pm 0.71\%$ *versus* $1.38 \pm 0.40\%$, $P < 0.01$). It was found that Th17 proportions in the KD-CAL$^+$ group were markedly higher than those in the KD-CAL$^-$ group ($2.81 \pm 0.74\%$ *versus* $2.06 \pm 0.53\%$, $P < 0.01$).

The frequencies of T$_{reg}$ (CD4$^+$CD25$^+$FoxP3^{high}/CD4$^+$ T cells) were significantly lower in the KD group compared with the NC group ($4.12 \pm 1.24\%$ *versus* $8.15 \pm 2.60\%$, $P < 0.01$). The frequencies of T$_{reg}$ cells in the ID group were almost equal to those in the NC group ($8.06 \pm 2.53\%$ *versus* $8.15 \pm 2.60\%$, $P > 0.01$). The proportions of T$_{reg}$ cells increased gradually after treatment with IVIG ($6.27 \pm 2.04\%$). There was no obvious difference between the KD-CAL$^+$ and KD-CAL$^-$ groups in the proportions of T$_{reg}$ cells ($P > 0.05$).

Expression of IL-17A/F, ROR-γt and FoxP3 in CD4$^+$ T from patients with KD

IL-17A/F is a prominent cytokine produced by Th17 cells. ROR-γt is an important transcription factor for the differentiation of Th17, while FoxP3 is the master transcription factor in T$_{reg}$. We thus investigated the expressions of ROR-γt and FoxP3 in CD4$^+$ T from patients with KD. As shown in Fig. 3, compared with the NC group, the levels of IL-17A/F and ROR-γt expression were up-regulated significantly in the ID group [IL-17A(8.42 ± 2.13) × 10^{-6} *versus* (3.82 ± 1.45) × 10^{-6}, $P < 0.01$, IL-17F (3.25 ± 0.87) × 10^{-4} *versus* (1.71 ± 0.50) × 10^{-4}, $P < 0.01$, ROR-γt (5.31 ± 1.35) × 10^{-5} *versus* (3.48 ± 1.03) × 10^{-5}, $P < 0.01$]. The levels of IL-17A/F and ROR-γt expression were much higher in the acute KD patient group than those in the ID group [IL-17A (17.56 ± 7.43) × 10^{-6}, IL-17F (7.96 ± 3.04) × 10^{-4}, ROR-γt (8.57 ± 2.65) × 10^{-5}] and remarkably lower after treatment with IVIG [IL-17A (10.68 ± 3.15) × 10^{-6}, IL-17F (3.07 ± 1.12) × 10^{-4}, ROR-γt (5.81 ± 1.68) × 10^{-5}, $P < 0.01$]. The levels of IL-17A/F and ROR-γt expression in the KD-CAL$^+$ group were markedly higher than those of the KD-CAL$^-$ group [IL-17A (20.00 ± 5.47) × 10^{-6} *versus* (15.88 ± 4.63) × 10^{-6}, $P < 0.01$, IL-17F (9.36 ± 3.01) × 10^{-4} *versus* (7.37 ± 2.15) × 10^{-4}, $P < 0.01$, ROR-γt (9.15 ± 2.82) × 10^{-5} *versus* (7.48 ± 2.11) × 10^{-5}, $P < 0.01$]. In contrast, the expression levels of FoxP3 mRNA was markedly lower in the KD group compared with the NC group [(3.69 ± 1.04) × 10^{-5} *versus* (17.84 ± 5.12) × 10^{-5}, $P < 0.01$], while there was no obvious difference between the KD-CAL$^+$ group and KD-CAL$^-$ group in the levels of FoxP3 expression ($P > 0.05$). The expression levels of FoxP3 mRNA in the ID group were almost equal to those in the NC group ($P > 0.05$).

Plasma cytokine concentrations in patients with KD

Plasma concentrations of IL-17A, IL-6, IL-23 and TGF-β were detected in each group (Table 5). The IL-17A, IL-6 and IL-23 concentrations in patients with KD were significantly higher compared with the concentrations in the NC and ID groups. It was found that serum IL-17A, IL-6 and IL-23 had a tendency to decrease after treatment with IVIG, but there was no significant difference in the concentrations of the cytokines before or after treatment with IVIG. Plasma TGF-β concentrations were markedly lower in the KD group than in the NC group ($P < 0.01$), while there was no obvious difference between the ID group and the NC group in concentrations of TGF-β ($P > 0.05$).

Plasma concentrations of IL-17A, IL-6 and IL-23 in the KD-CAL$^+$ groups were significantly higher than those of the KD-CAL$^-$ group, but there was no obvious difference to be found in concentrations of TGF-β between the KD-CAL$^+$ and KD-CAL$^-$ groups.

Alteration of Thl7 cells in IVIG-resistant KD

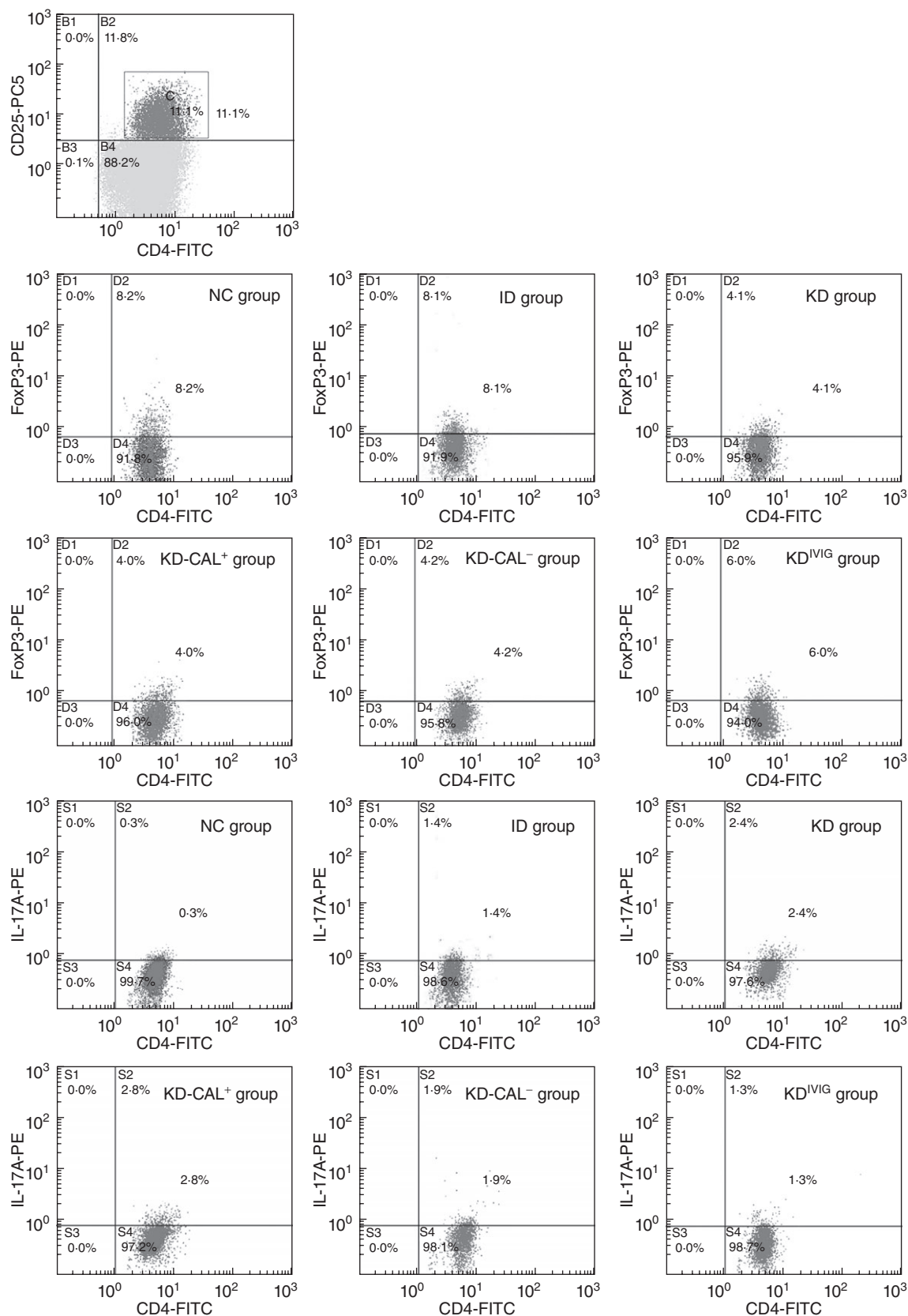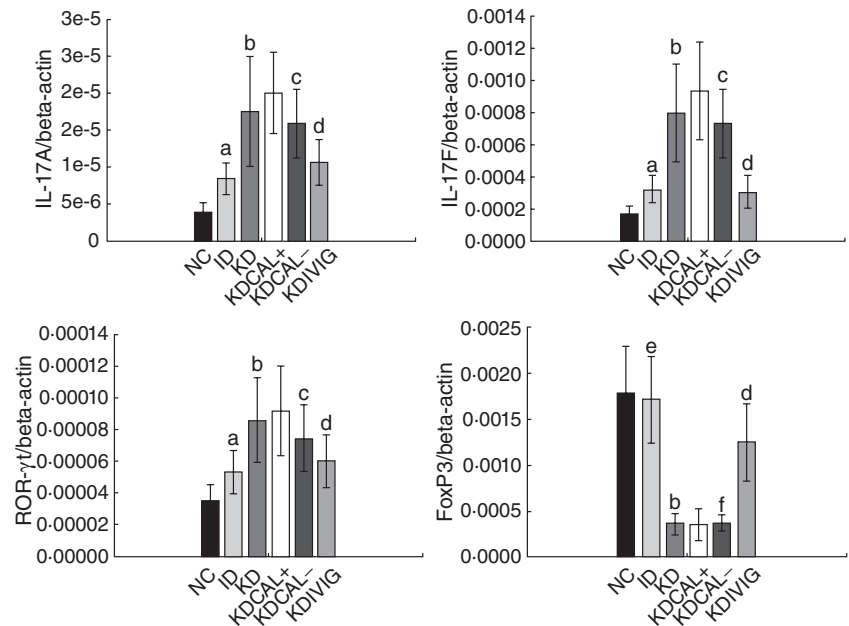Compared with the IVIG-sensitive group, the proportions of Th17 cells were up-regulated significantly during the

Fig. 2. The proportion of regulatory T cells (T_{reg}) and T helper type 17 (Th17) cells in patients with Kawasaki disease.

Fig. 3. Expression of transcription factors and cytokines in patients with Kawasaki disease; NC (normal controls) *versus* infectious disease (ID): [a]$P < 0.05$; ID *versus* KD: [b]$P < 0.05$, [e]$P > 0.05$; KD–coronary artery lesion (CAL[+]) *versus* KD-CAL[−]: [c]$P < 0.05$, [f]$P > 0.05$; KD *versus* KD[IVIG]: [d]$P < 0.05$.

acute phase in IVIG-resistant KD ($4.53 \pm 1.42\%$ *versus* $2.72 \pm 0.71\%$, $P < 0.01$).

Plasma concentrations of IL-17A and IL-6 in IVIG-resistant KD were found to be higher in comparison with the sensitive group before IVIG treatment (IL-17A 62.5 ± 18.4 pg/ml *versus* 30.3 ± 10.2 pg/ml, $P < 0.01$; IL-6 180.6 ± 50.5 pg/ml *versus* 71.6 ± 21.9 pg/ml, $P < 0.01$). The IVIG-resistant group was found to maintain continuously high levels of IL-17A IL-6 after IVIG treatment. There was no significant difference in the severity of decrease between both groups, but it was found that plasma concentrations of IL-17A and IL-6 had a tendency to decrease after treatment with IVIG (IL-17A 48.3 ± 14.5 pg/ml *versus* 28.1 ± 8.5 pg/ml, $P > 0.05$; IL-6 150.1 ± 46.7 pg/ml *versus* 65.9 ± 18.2 pg/ml, $P > 0.05$).

Discussion

KD is currently recognized as an acute vasculitis led by immune dysfunction disorder with cytokine cascade and endothelial cell lesion. Most research regarding cytokine networks in KD has focused upon inflammatory cytokines, such as IL-1, IL-2, IL-6, IL-8, interferon (IFN)-α and TNF-α [1–5]. These inflammatory cytokines are elevated during the acute phase of KD, and their changes parallel the clinical presentation of the systemic vasculitis, but the mechanism triggering the cascade response of proinflammatory cytokine production needs further clarification.

Recently, the Th17 cell has a lineage that is distinctly different from that of the Th1 and Th2 cells, which regulate tissue inflammation [7]. Its identification emerges from the discovery of a new type of cytokine: IL-17A/F [8]. IL-17 is a highly inflammatory cytokine with robust effects on stromal cells in many tissues. IL-17 produced by Th17 cells induces activation of neutrophils, stimulates monocytes and fibroblasts to produce proinflammatory cytokines such as IL-6, TNF-α, IL-8 and IL-1β, thus perpetuating inflammation of the tissues [8]. Therefore, Th17 cells and their effector cytokines may contribute to the pathogenesis of inflammation, autoimmune diseases and graft-*versus*-host disease (GVHD) [9]. T_{reg} cells have been demonstrated efficiently in the control of autoimmune disease. T_{reg} cells exert their function partly through the secretion of anti-inflammatory cytokines (IL-10 and TGF-β) [6]. The number of T_{reg} cells decreases in several autoimmune diseases, and adoptive transfer of purified T_{reg} cells improves autoimmune disorders [10,11]. Recent data in humans suggest that Th17/T_{reg} cells play an important role in the pathogenesis of a diverse group of immune-mediated diseases, including psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and asthma [12,13].

Sohn *et al.* have reported that IL-17 and IL-17-induced cytokines (IL-6 and IL-8) increased significantly in KD patients [14]. Several studies have reported that the proportions of T_{reg} cells are decreased and their functional properties compromised in patients with KD [15,16]. In this study, we observed Th17/T_{reg} cells on different levels, including cell proportions, related cytokine secretion and key transcription factors. Th17 proportions and expression levels of cytokines (IL-17A/F) and transcription factors (ROR-γt) were up-regulated significantly, while T_{reg} proportions and expression levels of T_{reg} transcription factor (FoxP3) were down-regulated significantly in children with acute KD. Although there was no obvious difference to be found in T_{reg} cells between the KD-CAL[+] and KD-CAL[−] groups, we found that Th17 proportions in the KD-CAL[+] group were markedly higher than those of the KD-CAL[−] group. Results mentioned

above indicate that a Th17/T$_{reg}$ cell imbalance exists in patients with KD, implicating a potential role for Th17/T$_{reg}$ imbalance in an acute vasculitis syndrome of KD.

In this study, we investigate further the proportions of Th17 cells in peripheral blood from IVIG-sensitive and -resistant patients. Compared with the sensitive group, Th17 proportions were up-regulated significantly during the acute phase in immunoglobulin-resistant KD. There was a significant difference in plasma concentrations of IL-17A and IL-6 between the resistant and sensitive groups before IVIG treatment. The resistant group still maintained significantly high levels of IL-17A and IL-6 after IVIG, suggesting that aberrant activation of Th17 cells may be one of the factors causing IVIG-resistant KD based on the inflammatory properties of Th17 cells.

Previous studies have shown that there is a reciprocal relationship between FoxP3$^+$ T$_{reg}$ and Th17 cells, in which IL-6 plays a pivotal role in dictating whether the immune response is dominated by pathogenic Th17 cells or protective T$_{reg}$ cells [13,16]. Naive T cells exposed to TGF-β up-regulate FoxP3 and become induced T$_{reg}$ cells, while Th17 cells are differentiated from naive T cells in response to IL-6 plus TGF-β, and need the presence of IL-23 for their expansion and/or maintenance [6,10,17,18]. In this study, we found that the concentrations of IL-6 and IL-23 in the patients with KD were significantly higher compared with the concentrations in NC and ID, while plasma TGF-β concentration was markedly lower in KD. Therefore, it can be speculated that over-expression of IL-6 might contribute to the Th17/ T$_{reg}$ imbalance in KD. However, the mechanisms causing disturbed expression of IL-6 and TGF-β need further investigation.

In summary, our data demonstrate that a Th17/T$_{reg}$ cell imbalance exists in patients with acute KD. Therefore, the dynamic interaction between Th17 and T$_{reg}$ cells may be important in the development of KD, suggesting a potential role for Th17/T$_{reg}$ imbalance in the pathogenesis of the KD acute vasculitis syndrome. Because of the reciprocal developmental pathway for the generation of Th17 and T$_{reg}$ cells, and their opposite effects, Th17/T$_{reg}$ subsets may have been evolved to induce or regulate tissue inflammation, analogous to the dichotomy of Th1/Th2 T cell subsets. We need to confirm our conclusion in a larger-scale population, and ongoing efforts should be made to identify the precise effect and mechanism of the Th17/T$_{reg}$ imbalance in KD. A better understanding of the nature, regulation and function of Th17 and T$_{reg}$ cells in KD immunity may provide a new target for the treatment of children with KD.

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Disclosure

None.

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